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# Gap Junctions among Taste Bud Cells in Mouse Fungiform Papillae

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## Introduction

Mouse taste buds in fungiform papillae consist of ~50 cells (TBCs; unpublished data), but only a few of them have synaptic contacts with taste nerves (Kinnamon *et al.*, 1993; Seta and Toyoshima, 1995). Neither chemical nor electrical synapses/gap junctions have been confirmed in mammalian taste buds, though a subpopulation of TBCs expressed a variety of neurotransmitter receptors (Kumazawa *et al.*, 2001; Hayato *et al.*, 2002). Non-innervated TBCs thus have been assumed to be supportive. However, we considered their roles in taste transduction.

We tested this hypothesis under *in situ* whole-cell patch clamp and optical recording conditions (Ohtubo *et al.*, 2001). Patch clamp studies showed that a part of TBCs generated depolarizing or hyperpolarizing receptor potentials in response to taste substances. Optical recordings with a voltage-sensitive dye showed that such chemosensitive TBCs tended to form colonies by the polarity of their receptor potentials, suggesting the existence of any interactions among TBCs.

In the present study, we show the diffusion of probe dyes from injected TBCs to their neighbors (dye-couplings), which evidences the existence of intercellular corridor between TBCs. These results suggest that there are TBC networks within mammalian taste buds, and that taste buds are miniature processing units rather than the aggregation of chemosensitive cells.

## Materials and methods

### Peeled lingual epithelia

We prepared peeled lingual epithelia as described previously (Furue and Yoshii, 1997, 1998) in accordance with Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences approved by the Council of the Physiological Society of Japan. In brief, we obtained tongues from mice etherized and then decapitated, subcutaneously injected a collagenase or elastase solution into tongues, peeled the epithelia with forceps ~5 min after the injection, and mounted on a recording platform (Figure 1). Under the conditions, the preserved epithelium and taste bud structures protected the basolateral membranes of TBCs as an effective barrier against deionized water and taste solutions such as high concentration of HCl or NaCl for >60 min (Furue and Yoshii, 1997, 1998).

### Detection of dye-couplings

We injected Lucifer Yellow CH (LY, 2 mg/ml) or biocytin (BC, 2 mg/ml) into TBCs through patch pipettes placed on the basolateral membranes of the TBC under in-situ voltage-clamp conditions (Figure 1). The peeled epithelia that contained LY-injected TBCs were examined either in fresh or after fixation. Those that contained BC-injected TBCs were fixed and then stained with streptavidin rhodamine red-X conjugate. The nuclei of TBCs in the fixed preparations were stained with acridine orange, a membrane permeable dye. The identification of nuclei within biocytin-coupled TBCs evidenced that stained structures were dye-coupled TBCs but not single complex TBCs, because TBCs are mononuclear.

We epi-illuminated the fresh preparations through a dichroic mirror unit, which reflected wavelengths between 400 and 440 nm, excited LY ($\lambda_{max}$ = 428 nm) through the 60× water-immersion objective and transmitted fluorescence through a high pass filter (>475 nm) to a cooled CCD camera. The fixed preparation was examined with a confocal laser microscope with similar filters.

## Results and discussion

### Dye couplings

Many cells absorb biocytin leaked from recording patch electrodes and yielded false biocytin-couplings. Although no TBCs absorbed biocytin when the electrode was placed 30 μm apart for 60 s, they

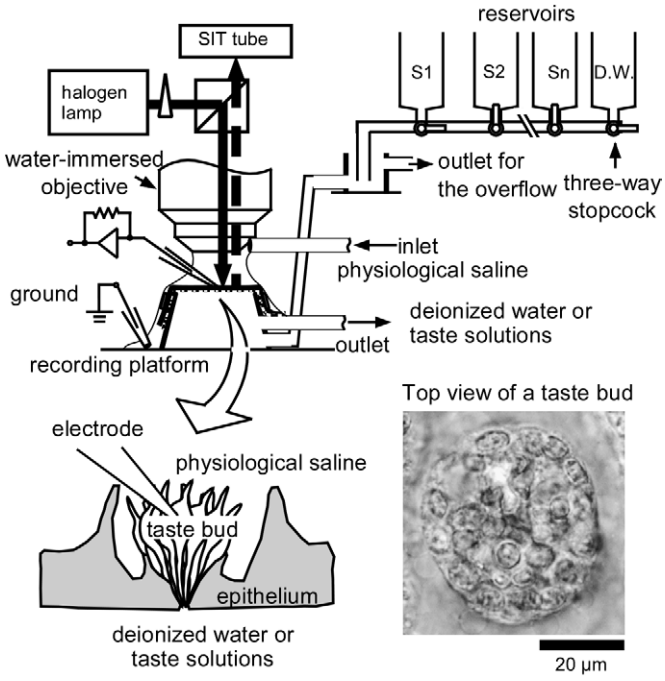


**Figure 1** Experimental set up for in-situ patch clamp and optical recordings. Peeled lingual epithelium mounted on a recording platform is placed under a microscope equipped with a water-immersed objective, where TBCs are injected with probe dyes under voltage-clamp conditions. The receptor membranes of TBCs facing inside the platform and their basolateral membranes facing the objective are irrigated separately. A photograph, a single TBC. Solid line, excitation path; broken line, fluorescence path. S1-Sn and D.W, reservoirs for stimulating solutions and deionized water, respectively.

absorbed it when the electrodes were placed 1 μm apart from them for >30 s. We eliminated such artifacts by forming giga-ohm seal in 10 s when recording electrodes left the 30 μm apart position. No TBCs absorbed LY.

The diffusion of biocytin from injected TBCs to another (biocytin-coupling) occurred in 10 TBCs of 56 examined and that of LY in five TBCs of 29 examined. There was no difference between biocytin-couplings and LY couplings in the occurrence ratio of dye-couplings. Single biocytin-couplings involved 2–5 TBCs with mean ± SD of 3.1 ± 1.2 ($n$ = 9). TBCs were different in morphology; rod, branched, flat and round types. Also, a group of TBCs extended their apical portions to the taste pore of respective taste buds and others did not. It is likely that dye-couplings occur irrespective of such morphological differences.

TBCs elicited various voltage-gated currents, such as TTX-sensitive $Na^+$ currents, TEA-sensitive outwardly rectifying $K^+$ currents, inwardly rectifying $K^+$ currents, $Cl^-$ currents, high-voltage and low-voltage activated $Ca^{2+}$ currents (Furue and Yoshii, 1997; Noguchi *et al.*, 2003). There are no differences between coupled and non-coupled TBCs in the magnitude of their pooled currents. Also, there were no differences in the pooled magnitude of membrane conductance and membrane capacitance.

### Junctional conductance

These results suggest that the junctional conductance of gap junctions is as low as membrane conductance. However, the occurrence of LY-couplings suggests that the junctional conductance is more than ~2 nS (Dermietzel and Spray, 1993). On the other hand, the membrane conductance of non-coupled cells was ~1 nS or less. Under whole-cell clamp conditions, the simplified equivalent electric circuit for coupled TBCs is a 2 nS conductor (junctional conductance) in series with a 1 nS conductor (membrane conductance of a coupled TBC). The total membrane conductance and capacitance must be higher than those of non-coupled TBCs.

The membrane conductance and membrane capacitance of TBCs were different from cell to cell. Averaging procedures seem to conceal the differences between coupled and non-coupled TBCs in the magnitude of these membrane properties. If it was possible to regulate the gating of gap junctions, statistic tests such as paired $t$-test would discriminate coupled and uncoupled TBCs.

### TBC networks

Dye-couplings (Sata *et al.*, 1992) and both dye-couplings and electrical couplings (Bigiani and Roper, 1993) showed gap junctions in amphibian TBCs. However, extensive electron microscopic studies have failed to confirm the occurrence of gap junctions in mammalian taste buds, though they had been reported 36 years ago in rat vallate papillae (Akisaka and Oda, 1978). Dye-couplings appear to be more sensitive in detecting gap junctions than electron microscopy. Gap junctions are thus found in both mammalian and amphibian taste buds. They may be fundamental devices in taste transduction.

In mouse fungiform papillae, only type III cells have synaptic contacts with taste nerve. It appears that type II cells transmit taste information to type III cells through gap junctions. When type III cells have neurotransmitter receptors, paracrine systems in addition to gap junctions can transmit taste information. Thus gap junctions together with paracrine systems contribute in forming TBC networks which process taste information (Figure 2).

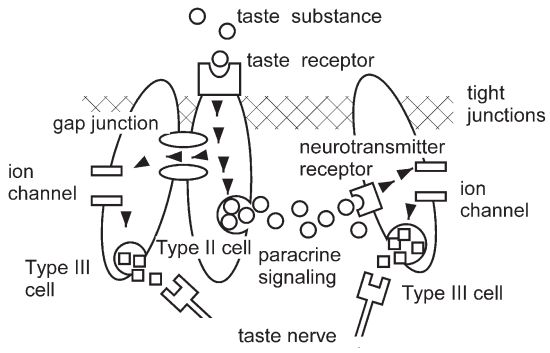


**Figure 2** Hypothetical TBC network formed by gap junctions and paracrine systems. Type II cell generates second messengers in response to taste substances. These second messengers diffuse into a type III cell through gap junctions, and elicit receptor potentials by gating ion channels on the cell, which release neurotransmitter and activate innervating taste nerves. Thee second messengers also generate receptor potential (not shown) and release neurotransmitters, which diffuse into interstitial spaces and activate neurotransmitter on another type III cell.

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